P–C5–17

SITE-DIRECTED SPIN LABELING AND CHEMICAL CROSS-LINKING DEMONSTRATE THAT HELIX V IS CLOSE TO HELICES VII AND VIII IN THE LACTOSE PERMEASE.

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Purpose: To determine the structure and mechanism of the lactose permease of *E. coli.*
Methods: Permease containing a biotin domain and paired Cys residues at positions 148 (helix V) and 228 (helix VII), 148 and 226 (helix VII) or 148 and 275 (helix VIII) was affinity purified and labeled with a sulfhydryl-specific nitroxide spin label. The EPR spectra at ambient temperature and in the frozen state were analyzed to determine the proximity of each pair of tethered spin labels. Also, dibromobimane is used to crosslink paired-Cys residues.
Results: Spin-spin interactions are observed with the 148/228 and 148/275 pairs. No interaction is evident with the 148/226 pair. Analysis of broadening of the EPR spectra in the frozen state yields inter-spin distances of approximately 9 Å and 14 Å for 148/228 and 148/275, respectively. Dibromobimane crosslinks paired Cys 148/228.
Conclusions: The results provide strong support for a structure in which helix V is in close proximity to both helices VII an VIII and oriented where Cys148 is closer to helix VII.

P–C5–18

Ca^{2+} REGULATION OF GABA TRANSPORT BY SPM VESICLES: POSSIBLE INVOLVEMENT OF CALCINEURIN ACTIVITY

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Purpose: The transport of γ-aminobutyric acid (GABA) by synaptic plasma membrane (SPM) vesicles is inhibited by the presence of Ca^{2+} inside the vesicles. So, we investigated the possible targets for the Ca^{2+} action in the regulation of the SPM GABA transport in response to elevation of intravesicular [Ca^{2+}].
Methods: SPM vesicles were isolated from osmotic disrupted sheep brain cortex synaptosomes by ultracentrifugation in a dextran T110 gradient. GABA uptake by SPM vesicles was measured isotopically by using [^{3}H]GABA and a filtration method.
Results: We tested wether trifluoperazine and okadaic acid alter the inhibitory effect of intravesicular Ca^{2+} on the GABA transport by SPM vesicles observed in response to artificially imposed ionic gradients.
Conclusions: Since 40μM trifluoperazine and 10 μM okadaic acid antagonize the Ca^{2+} effect, we conclude that calmodulin dependent phosphatase activity of calcineurin may be involved in the Ca^{2+} regulation of the neuronal GABA transport.

P–C5–19

PURIFICATION AND RECONSTITUTION OF THE GLUTAMATE TRANSPORTER OF *BACILLUS STEAROTHERMOPHILUS.*

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Purpose: The cation selectivity of the glutamate carrier of *B. stearothermophilus* depends on the host in which it is expressed. In membrane vesicles of *B. stearothermophilus* one Na^+ and one H^+ are cotransported with glutamate, while in *E.coli* vesicles two protons are used. Differences in lipid environment may be responsible for this discrepancy.

Methods: The purified protein is reconstituted in liposomes composed of different phospholipids in order to study the influence of the lipid environment on the cation selectivity of the carrier.

Results: The carrier, fused to a N-terminal His-tag, was expressed in *E. coli* and could be purified from the solubilized membranes in one step using Ni^{2+}-affinity chromatography. The carrier was actively reconstituted in liposomes derived from *E. coli* lipids and was shown not to use Na^+ as coupling ion.

Conclusions: In liposomes composed of *E. coli* lipids the purified glutamate carrier from *B. stearothermophilus* uses only protons as coupling ions. Attemps are now underway to reconstitute the carrier in liposomes composed of lipids from *B. stearothermophilus.*

P–C5–20

CYCLAM LIPIDS AS MEMBRANE-BOUND CHELATORS

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Purpose: We have synthesized N-hexadecyl cyclam (HDC) and N,N'-dihexadecyl cyclam (DHDC) and studied the lipid-mixing properties of these long-chain derivatives of cyclam.
Methods: The behavior of the mixed-lipid systems HDC or DHDC with DMPC was investigated using differential scanning calorimetry (DSC) and fluorescence spectroscopies.
Results: HDC showed no thermal transition between 5 - 95 °C, whereas DHDC had both a pre-transition and a main transition temperature. Both fluorescence and DSC show that HDC/DMPC mixed in with DMPC with the narrowest mixed-phase region at 50 mol%. DHDC also broadened the phase transition of DMPC. Both kinds of data indicate that the derivatives are mixing into the bilayer, satisfying the criterion for membrane-bound chelation.
Conclusions: These compounds are potentially useful for selectively chelating heavy-metal cations onto lipid membrane surfaces.